

SYNTHESIS, SPECTROSCOPIC, THERMAL AND BIOLOGICAL STUDIES of SOME NOVEL METAL IONS COMPLEXES

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Abstract:

In the current study, a novel heterocyclic azo ligand, 4-[6-(p-phenyl phenol)azo] cytosine(PAC), is synthesized through a general diazo coupling reaction with lanthanide ions [La(III), Nd(III), and Eu(III)]. The molecular formulae of the newly synthesized compounds were established using a variety of spectroscopic methods, including [FT-IR, UV-Vis, and HNMR], elemental analysis (C.H.N), thermal gravimetric analysis (TGA), magnetic measurement, molar conductivity measurement, stability constant, and Gibbs free energy. Using the mole ratio method, the complexes stoichiometry was determined using data from spectroscopic analyses of the complexes' solutions for the chosen ions. The metal to ligand mole ratio, however, was (1:2). When the biological effectiveness of each compound was tested against various bacteria and fungi strains, the results showed good activity when compared to the standard medication. additionally possess effective cancer prevention and dyeing performance.

Keywords: Cytosine, Azo Dye, Cytotoxicity, Apoptosis.

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Introduction:

Pyrimidine compounds are received wide interest in biological systems exists in nucleic acids construct in living systems, coenzymes, many vitamins and biotics, as well as have therapeutic importance [1]. The existence of different chelating sites in the geometry of this type of compound sleds to utilize as ligand with metal ions. At the same time metal ions have been widely utilized in pharmaceutical filed for many diseases such as chemotherapeutic agent in the treatment of many kind malignancies such as liver , lung, breast cancers. Due to virtue of their ability to modulate many biological modes involving DNA. The chelating complexes have been extradited a considerable attention as anticarcinoma agent [2]. Cytosine is a nucleic base and most important pyrimidine derivatives, which have therapeutic importance [3]. The importance of nitrogen bases in the living systems lies in entry into metabolic processes[4]. Nucleotide and derivatives of nitrogen bases play on important roles in protection reactions and cell division[5] and also have an important biological activity such as antifungal , anti-bacterial , antiviral, anticancer and antioxidant due to ability to inhibit cell growth pathogenesis[6]. On the other hand, azo compounds attracted the attention of many researchers due to its many uses in the medical field, they are used as medicine [7]. They displayed antioxidant , anticancer , antiviral , antimicrobial and antifungal activities [6]. In addition to these utilize azo compounds are also utilized in analytical, industrial and ligands in chelating chemistry[8]

In this research, the synthesis and characterization of azo ligand derived from cytosine and it's lanthanide complexes with studies the medical properties.

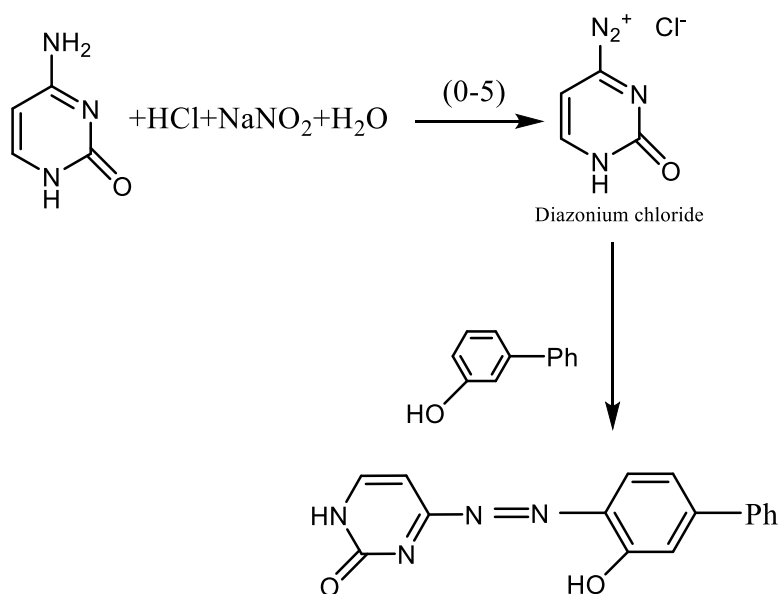
Experimental:

Instruments and materials

Analysis and metal content of the ligand and its complexes are determined using (C.H.N.S) (Eure EA 3000 Elemental analyzer). pH meter JENWAY 3020 FT-IR spectrophotometry is a type of spectrophotometry that employs the Fourier Series Infrared Transform. The SHIMADZU 8400s spectrophotometer was used to record infrared spectra with CsI in the range (250- 4000) cm^{-1} . The (SHIMADZU 1800 - UV-Vis spectrophotometer) was used to record UV-Vis spectra for all of the substances tested in the (250-1100) nm range. The ^1H -NMR spectra were measured on a BRUKER AV 400 Avance-III (400 MHz and 100 MHz). analyses and metal content of the ligand and its complexes are available. Thermal analysis (TGA and DSC) was used to assess the metal content of the synthesized ligands and complexes (SDT Q600 V20.9 Build). The melting points of each compound were calculated using Gallenkamps melting point device. The molar conductance of metal ion complexes was studied in deionized distilled water (10⁻³ M). The chloride content of the complexes under investigation was ascertained using the Mohr method. A Sherwood Scientific Auto Magnetic Susceptibility Balance Model was used to measure the investigated complexes' magnetic susceptibility at room temperature.

Synthesis of the ligand 4-[6-(P-phenyl phenol) azo] cytosin (PAC)

The ligand (PAC) was synthesized in the following manner as in the literature [8] in some ways [scheme(1)]. A solution of cytosine (0.01 mole, 1.1110 gm) and 37 percent hydrochloric acid (0.2 mole, soluble in 25 ml of distilled water) was created, and the resulting substance was then kept in an ice bath at a temperature of (0 to 5) $^{\circ}\text{C}$. Then, (0.2 mole) of sodium nitrite in (25 ml) distilled water was added dropwise to the amine hydrochloride with constant stirring for one hour to create diazonium chloride. This solution was then combined with a cooled alkaline ethanolic solution of (0.01 mole, 1.700gm) m-phenyl phenol, and the crude azo ligand was collected by filtration, crystallized with (1:1) (H_2O :EtOH) and washed with acetone.



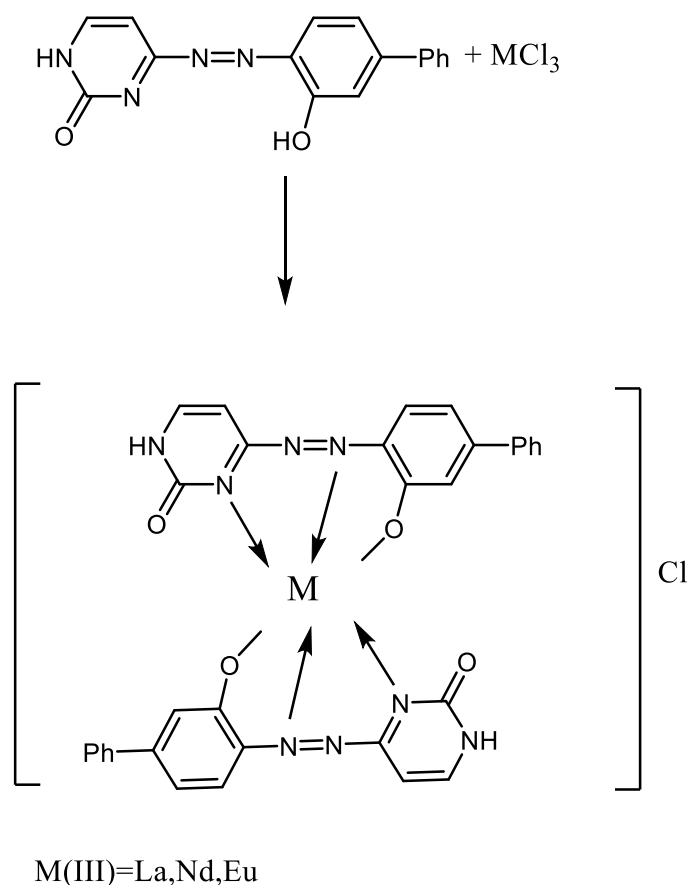
Scheme (1): Synthesis of ligand (PAC)

Determination of the stoichiometry of the complex solutions (Mole ratio method)

The absorption of numerous metal: ligand solutions was determined using the mole ratio method [9] in order to determine the mole ratio (M:L). The absorption spectra of numerous mixed solutions containing 1 ml of the metal ion salt and varying volumes (by increasing 0.25 ml) of the ligand solution at the same concentration were measured. The (M:L) ratio was calculated by plotting the relationship between the mole ratio on the x-axis and the absorbance on the y-axis, where the intersection of the two straight lines represents the (M:L) ratio.

Synthesis of the complexes [General procedure]

lanthanide complexes were synthesized in a (M:L) ratio (1:2) by dissolving (2mmole 0.584gm) of the ligand (PAC) in minimum quantity of ethanol the ethanoic solution of the ligand (PAC) was added gradually with stirring to the selected lanthanide salt containing an accurate amount of each metal chloride (1mmole , 0.2452 gm , 0.2505 gm and 0.3664 gm) LaCl_3 , $\text{NdCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$, respectively. The each mixture was stirred at room temperature for (1hr) and the reaction was mentioned by (TLC) technique [9]. The color precipitate was filtered off and dry [scheme (2)].

**Scheme(2): Synthesis of complexes****Antimicrobial effectiveness**

Novel azo ligand (PAC) and its complexes were screened in vitro with four species of bacteria and one species of fungi by microdilution method calculation MIC (minimal Inhibition concentration) [10^{-3}M] ethanol [10]. The inhibition zones (mm) were scaled after 48hr old cultures of selected bacteria were spread in 20ml agar in petri dish and incubated (24hr) at 37°C for bacteria and (72hr) for fungi, with Ciprofloxacin and Clotrimazole as a control for bacteria and fungi respectively.

Anticancer effectiveness

Toxic effect of the $[\text{Nd}(\text{PAC})_2]\text{Cl}$ complex was assessed against lung cancer line (A549) and normal cell line (WRL68) by MTT assay [11]. The cancer cells were cultured in (96-well plate) consist at minimum essential media with (10%) inactivated fetal calf serum concentrations of the complex $[\text{Nd}(\text{PAC})_2]\text{Cl}$ after incubation for (24hrs, and 72hrs at 37°C) with concentration of (10, 20, 30, 50, and 100) $\mu\text{g/ml}$ were prepared.

Dying method:

The ligand (PAC) and its complexes were utilized as a dyes to dying the wool fabric. (0.25 gm) of dyes were dissolved in an ethanoic solution of (10%) sodium hydroxide, after that added (50) ml distilled water. A piece of clear white wool fabric was soaked in distilled water for (30) minutes then transferred to the azo dye solution and heated to $(60)^\circ\text{C}$ to (10) minutes. The

cotton piece was washed many times with distilled water to remove any unreacted dye. Finally, dried by hot steam.

Result and Discussion:

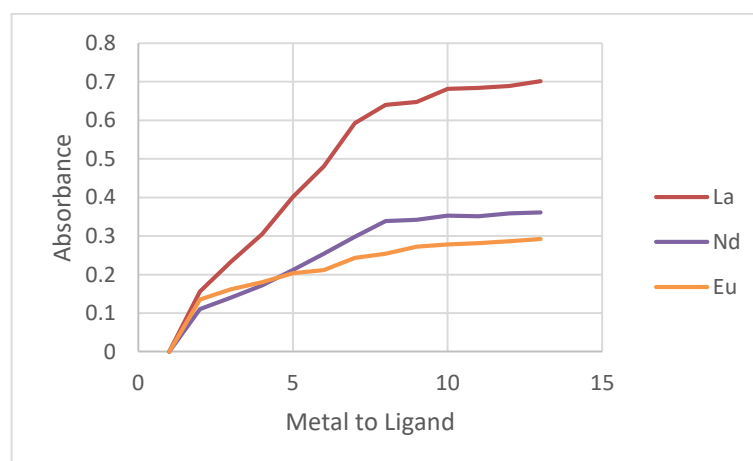
The synthetic route taken for the synthesis of novel azo ligand (PAC) and its Ln-complexes having cytosine core is depicted in scheme (1) and (2) all physico properties were summarized in the Table (1). The ligand was synthesized by coupling of m.phenylphenol (as nucleophile) with cytosine as diazonium salt (electrophile) at (0-5) °C. The synthesized compound were isolated as colored solids, that are stable at room temperature and towards atmosphere. They are soluble in common organic solvent like EtOH , MeOH, DMSO, DMF,... etc. All complexes have ionic characters so were observed from their molar conductivity result in (10⁻³M) in EtOH they have (1:1) electrolyte by presence chloride ion as counter ion [12].

Table (1): Physicochemical Properties For The Ligands (PAC) And Their Complexes

Compound M.Wt. (gm/mole)	M:L	Color $\lambda_{\max}$ nm	Elemental analysis experimental % (Theoretical)%					Λ_m ohm ⁻¹ .cm ² .mol ⁻¹
			C	H	N	M	Cl	
PAC C ₁₆ H ₁₂ N ₄ O ₂ (292)	–	Orange 441	66 (65.75)	4.28 (4.10)	19.88 (19.17)	–	–	–
LaC ₃₂ H ₂₂ N ₈ O ₄ CL (756.405)	1:2	Purple 541	51.01 (50.71)	3.45 (2.96)	14.92 (14.80)	18.93 (4.69)	5.00 (4.69)	38
NdC ₃₂ H ₂₂ N ₈ O ₄ CL (761.75)	1:2	Red 481	50.02 (50.71)	3.08 (2.88)	14.95 (14.70)	19.11 (18.93)	5.02 (4.66)	41
EuC ₃₂ H ₂₂ N ₈ O ₄ CL (769.50)	1:2	Violet 554	48.56 (48.21)	2.87 (2.76)	14.88 (14.51)	20.01 (19.70)	4.80 (4.60)	36

Nature of the complexes

The stoichiometric interaction of the ligand (PAC) and its complexes were studied by applying the mole ratio method. This method was measured absorbance was plotted against molar ratio by increase (0.25) ml for ligand while the volume metal ion remain constant. Figure(1) was shown that mole ratio for all studied complexes are (1:2) at $\lambda_{\max}$.



Figure(1) : Mole Ratio Plot For The PAC. Complexes Solutions At $\lambda_{\max}$.

Stability constant and Gibbs free Energy

The stability constant (K) for lanthanide complexes, when the (M:L) ratio is (1:2) was numbered depending to the equations (1) and (2) [13]

$$K = \frac{1-\alpha}{4\alpha^2 C^2} \dots \dots (1) ; \alpha = \frac{A_m - A_s}{A_m} \dots \dots (2)$$

Where: A_m = Absorbance of solution containing excess of ligand

A_s = Absorbance of solution containing a stoichiometric amount of ligand and metal ion

C = molar concentration (*gm/mole*)

All results were listed in the Table (2). The high value at K indicate high stability of complex.

Gibbs free energy (ΔG) was also studied from the equation [3] (14)

$$\Delta G = -RT \ln K \dots \dots (3)$$

Where: R = Gas constant = $8.3 \text{ J.mole}^{-1} \cdot \text{K}$.

T = Absolute Temperature (Kelvin)

All data was appeared that the reaction between the ligand (PAC) and the selected lanthanide ions are spontaneous [Table (2)]

The electronic spectra and Magnetic susceptibility:

The electronic spectra of the ligand (PAC) and their selected lanthanides complexes were recorded in ethanol [10^{-4} M] in the range [250-1100] nm Figures (2-5)] and their assignments were tabulated in the Table (2). The electronic spectra of the ligand (PAC) was displayed two band at (325 and 441) nm due to ($\pi \rightarrow \pi^*$) transition for aromatic moiety and ($n \rightarrow \pi^*$) transition of intramolecular charge transfer (Intra-CT) taken place through the azo moiety [15].

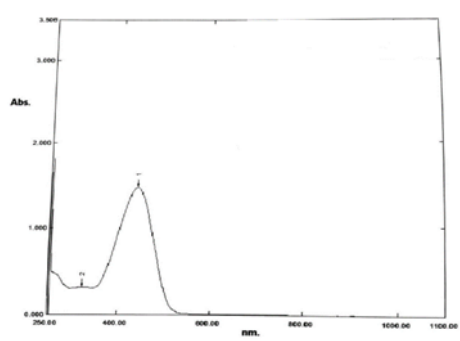
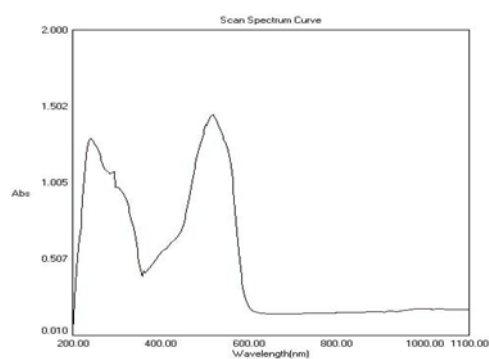
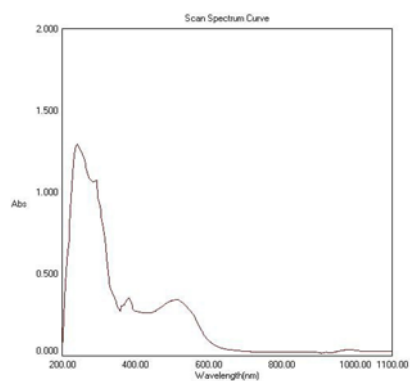
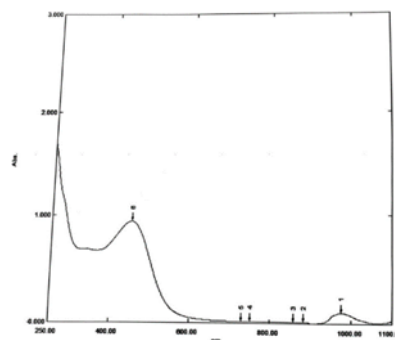
The electronic spectra of complexes were investigated and appeared great bathochromic shift (40-113) nm, which was revealed accompanied with color change from orange to red or purple or violet, this is strongly indicating the interaction of ligand with the lanthanide ions.

The (f-f) transitions for the electron in (4f) subshell partly filled of lanthanide ions are only slightly crystal-field affected by encirclement of lanthanoid ion due to (4f) electrons lie deep inside the (5s and 5p) electrons, that can't overlap easily with ligand orbitals and thus, it is not part of the coordination. Then have little effect on the spectral bands [13,16].

The magnetic properties of the synthesized complexes are listed in Table (2). The magnetic moment of (f-block) elements were difficult to explain due to (4f) electrons were inside the core (5s and 5p) electrons [16].

Table (2): Electron Spectra, Stability Constant, Gibbs Free Energy And Magnetic Susceptibility

Compound	λ_{max} (nm)	Wave number (cm^{-1})	Assignment	Lnk	ΔG $\text{J.mol}^{-1}.\text{k}$	μ B.M
(PAC)	441 325	22675 30769	$n \rightarrow \pi^*$ ($\pi \rightarrow \pi^*$)	— —	— —	— —
$[\text{La}(\text{PAC})_2]\text{Cl}$	541	18484	CT	14.845	-36717.62	0.00
$[\text{Nd}(\text{PAC})_2]\text{Cl}$	481	20790	CT	14.854	-36739.88	3.51
$[\text{Eu}(\text{PAC})_2]\text{Cl}$	554	18050	CT	16.252	-40199.61	3.65

**Figure(2): UV-Vis Spectrum for the (PAC) ligand****Figure (3):UV-Vis Spectrum for the $[\text{Eu}(\text{PAC})_2]\text{Cl}$ complex****Figure (4):UV-Vis Spectrum for the $[\text{La}(\text{PAC})_2]\text{Cl}$ complex****Figure(5):UV-Vis Spectrum for the $[\text{Nd}(\text{PAC})_2]\text{Cl}$ complex**

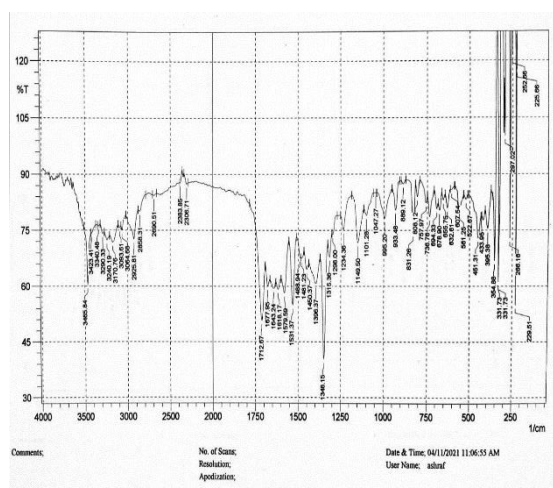
FT-IR Spectral data

FT-IR spectral of the ligand and its complexes were recorded as CsI pellets in the region (4000-200) cm^{-1} [Figures(6-8)]. The important bands were demonstrated in Table(3). The spectrum of the ligand was compared to those of the selected lanthanide complexes in order to assert the binding mode of the ligand to the corresponding lanthanide ion in the complex. Sharp absorption band was shown at (3465) cm^{-1} assigned to $\nu(\text{OH})$ of phenol moiety in ligand spectrum this band was disappeared in the spectra of all complexes indicating that the hydroxyl moiety is involvement in chelating ring [13]. The $\nu(\text{C}=\text{O})$ of amide[17] was exhibited at (1712) cm^{-1} in the ligand (PAC) spectrum. No change in intensity and position was noticed in the spectra of complexes, which indicated that this moiety not coordinated with the lanthanide ion. While $\nu(\text{C}=\text{N})$ and $\nu(\text{N}=\text{N})$ were appeared significant change in the stretching frequency and shape due coordination *via* nitrogen of azo moiety and nitrogen of pyrimidine moiety [18]. On the other hand new bands were noticed in the spectra of the complexes belong to the $\nu(\text{M}-\text{N}_{\text{prm}})$, $(\text{M}-\text{N}_{\text{azo}})$, and $\nu(\text{M}-\text{O})$ [15,19]. Which indicating that the ligand (PAC) behaves as negative tridentate through nitrogen of pyrimidine moiety, nitrogen of azo moiety, and oxygen of ortho hydroxyl moiety respectively.

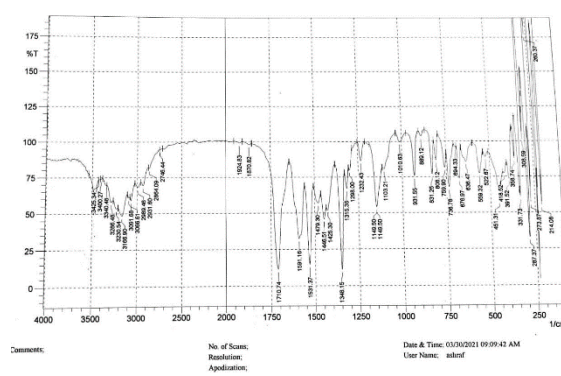
Table(3): FT-IR Spectral data (cm^{-1}) of the ligand (PAC) and its complexes

Assignment	PAC	$[\text{La}(\text{PAC})_2]\text{Cl}$	$[\text{Nd}(\text{PAC})_2]\text{Cl}$	$[\text{Eu}(\text{PAC})_2]\text{Cl}$
$\nu(\text{OH})$	3465sh	----	----	----
$\nu(\text{C}=\text{O})$	1712sh	1710sh	1714sh	1712sh
$\nu(\text{C}=\text{N})$	$\left. \begin{matrix} 1677 \\ 1643 \\ 1618 \\ 1579 \end{matrix} \right\} m$	1591st	$\left. \begin{matrix} 1612 d \\ 1579 m \end{matrix} \right\}$	1580m
$\nu(-\text{N}=\text{N}-)$	$\left. \begin{matrix} 1450 \\ 1396 \end{matrix} \right\} m$	$\left. \begin{matrix} 1446 d \\ 1425 m \end{matrix} \right\}$	$\left. \begin{matrix} 1448 d \\ 1427 w \end{matrix} \right\}$	$\left. \begin{matrix} 1440 d \\ 1422 w \end{matrix} \right\}$
$\nu(\text{M}-\text{O})$	----	559w	561w	550w
$\nu(\text{M}-\text{N}_{\text{azo}})$	----	451w	418w	430w
$\nu(\text{M}-\text{N}_{\text{prm}})$	----	391w	364vw	395vw

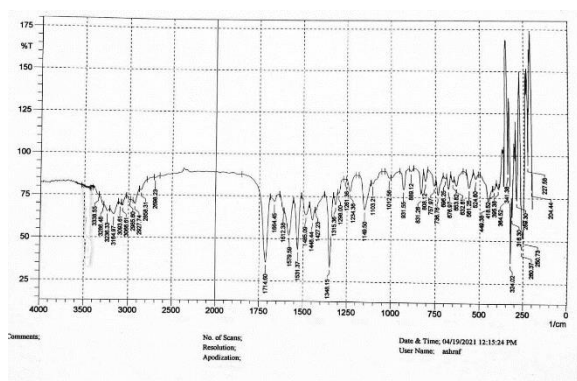
Sh=sharp , st=strong , d=doublet ,m=medium m w=weak ,vw=very weak.



Figure(6): FTIR spectrum of PAC ligand.



Figure(7):FTIR spectrum of [La(PAC)₂]Cl complex

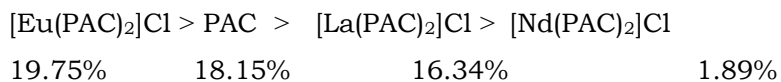


Figure(8):FTIR spectrum of [Nd(PAC)₂]Cl complex

Thermal Analysis

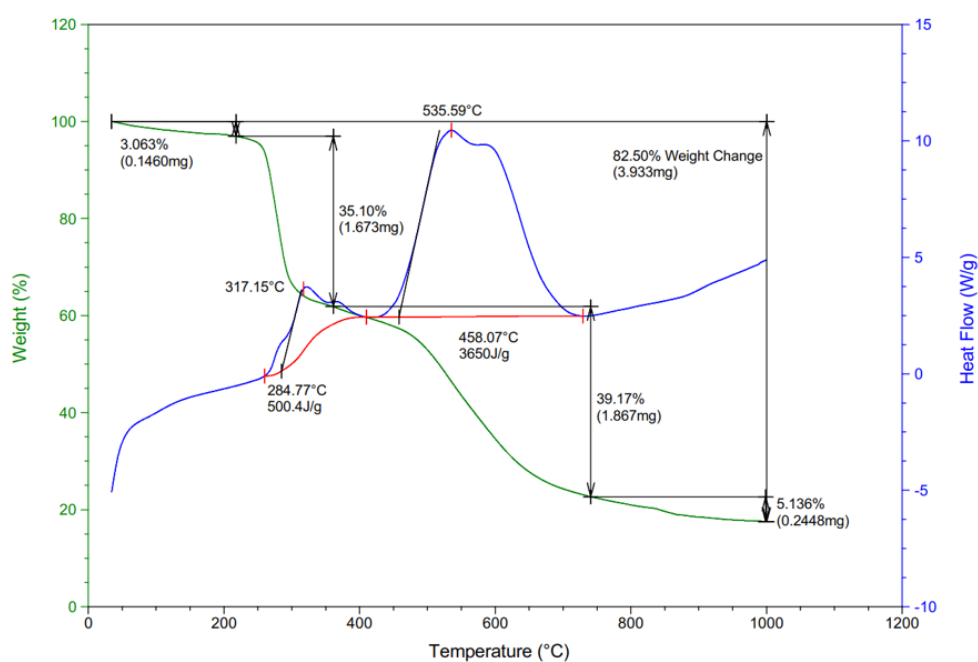
Thermal behavior of the ligand (PAC) and its complexes were investigated by thermograms (DSC-TG) [Figures(9-12)] and the corresponding thermal analysis result listed in the Table (4) with temperature range (25-1000)°C in air. In the case of the ligand (PAC), the decomposition was taken place in four exothermic steps. There is no mass loss up (240) °C. The mass loss at first step is (3.063%), which accompanied by exothermic effect in the DSC thermogram. The rest of the step are listed in the Table (4). In case of complexes they all no mass loss up to 200°C in which was indicated there is no lattice water in crystal structure of

the complexes [21].The thermal stability of the ligand and its complexes depending to the percentage of the residue[22] is :-

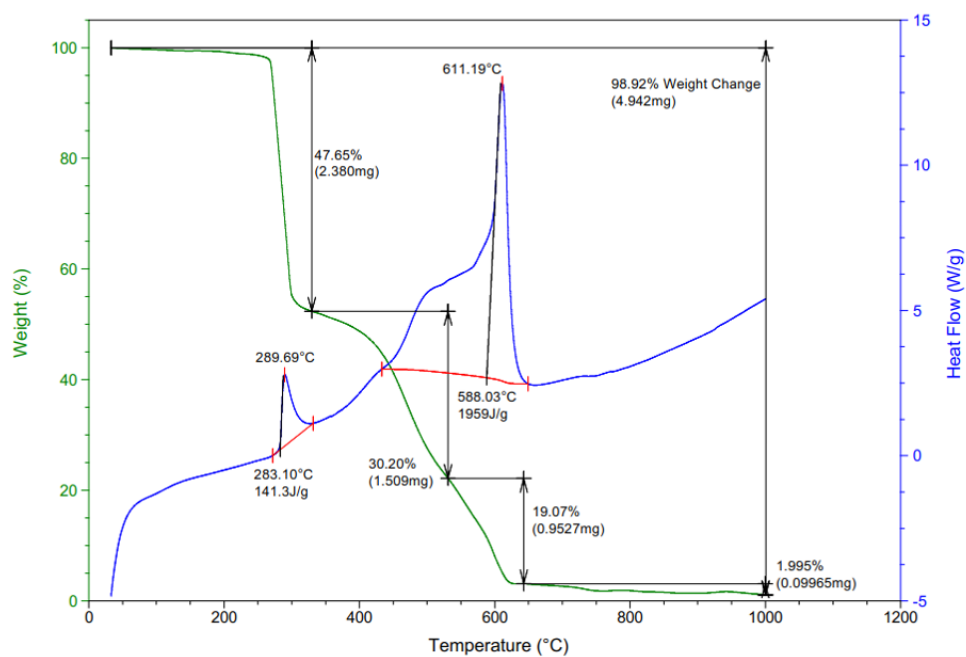


Compound symbol	Molecular formula (mw)gm/mole	step	TG-rang of the decomposition	Suggested Assignment	Mass loss%		DSC °C
					calculate	found	
PAC	$\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}_2$ (292)	1	25-240	$\text{C}_{0.5}\text{H}_3$	3.08	3.06	317.15 EXO
		2	240-360	C_8H_6	34.93	35.10	535.59 EXO
		3	360-775	$\text{C}_{7.5}\text{H}_3\text{N}_{1.5}$	39.04	39.17	----
		4	775-1000	N	4.79	5.136	----
		residue	>1000	$\text{N}_{1.5}\text{O}_2$	18.15	17.50	----
$[\text{La}(\text{PAC})_2]\text{Cl}$	$\text{LaC}_{32}\text{H}_{22}\text{N}_8\text{O}_4\text{Cl}$ (756.405)	1	25-225	CH_8	2.64	2.76	274.89 EXO
		2	225-380	$\text{C}_{10}\text{H}_{12}$	17.45	17.46	472.58 EXO
		3	380-565	$\text{C}_{21}\text{H}_2\text{N}_4$	45.67	46.41	----
		4	565-1000	$\text{La}_{0.1}\text{N}_4\text{O}_4$	17.88	18.01	----
		residue	>1000	$\text{La}_{0.9}$	16.34	15.29	----
$[\text{Nd}(\text{PAC})_2]\text{Cl}$	$\text{NdC}_{32}\text{H}_{22}\text{N}_8\text{O}_4\text{Cl}$ (761.75)	1	25-330	C_{30}H_3	47.65	47.65	289.69 EXO
		2	330-540	$\text{C}_2\text{H}_{19}\text{N}_8\text{ClO}_{2.3}$	29.83	30.20	611.19 EXO
		3	540-640	$\text{Nd}_{0.8}\text{O}_{1.7}$	18.71	19.07	----
		4	640-1000	$\text{Nd}_{0.1}$	1.89	1.99	----
		residue	>1000	$\text{Nd}_{0.1}$	1.89	1.08	----
$[\text{Eu}(\text{PAC})_2]\text{Cl}$	$\text{EuC}_{32}\text{H}_{22}\text{N}_8\text{O}_4\text{Cl}$ (769.5)	1	25-350	$\text{C}_{16}\text{H}_{20}\text{Cl}$	32.16	32.15	307.55 EXO
		2	350-600	$\text{C}_{16}\text{H}_2\text{N}_{3.5}$	31.57	32.02	499.29 EXO
		3	600-1000	$\text{N}_{4.5}\text{O}_4$	16.50	16.98	----
		residue	>1000	Eu	19.75	18.66	----

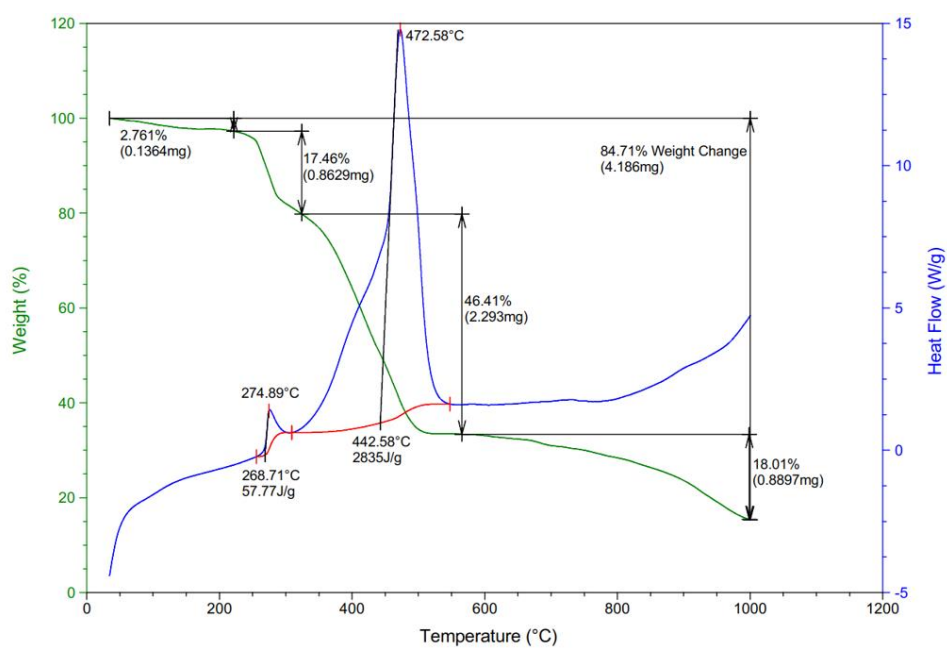
Table(4): Thermal Analysis of ligand it's complexes



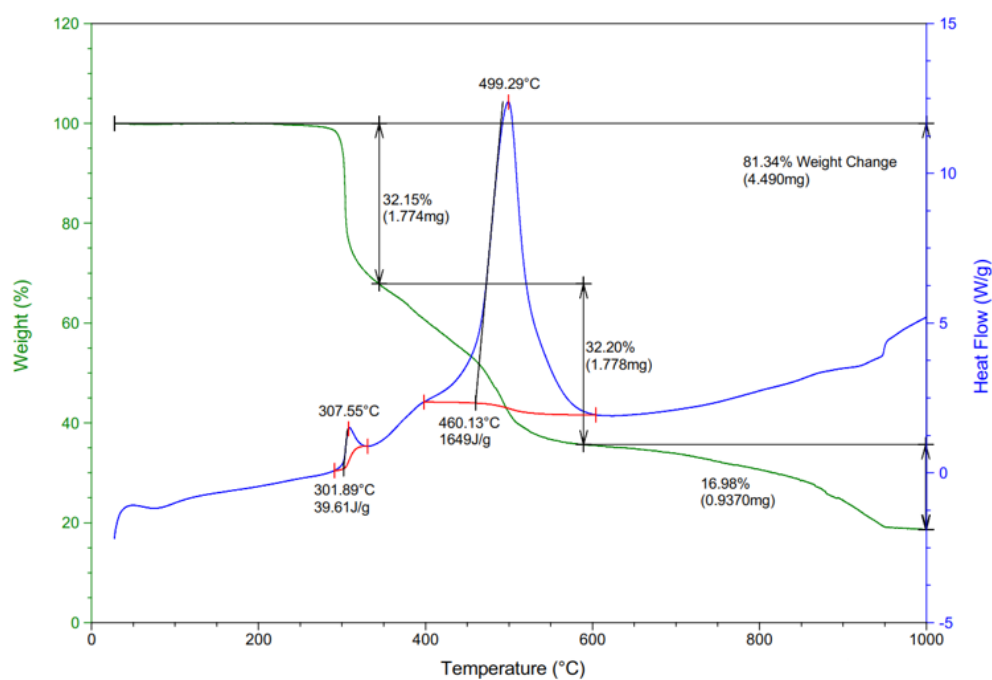
Figure(9):Thermogram for the [(PAC)]ligand



Figure(10):Thermogram for the $[\text{Nd}(\text{PAC})_2]\text{Cl}$ complex



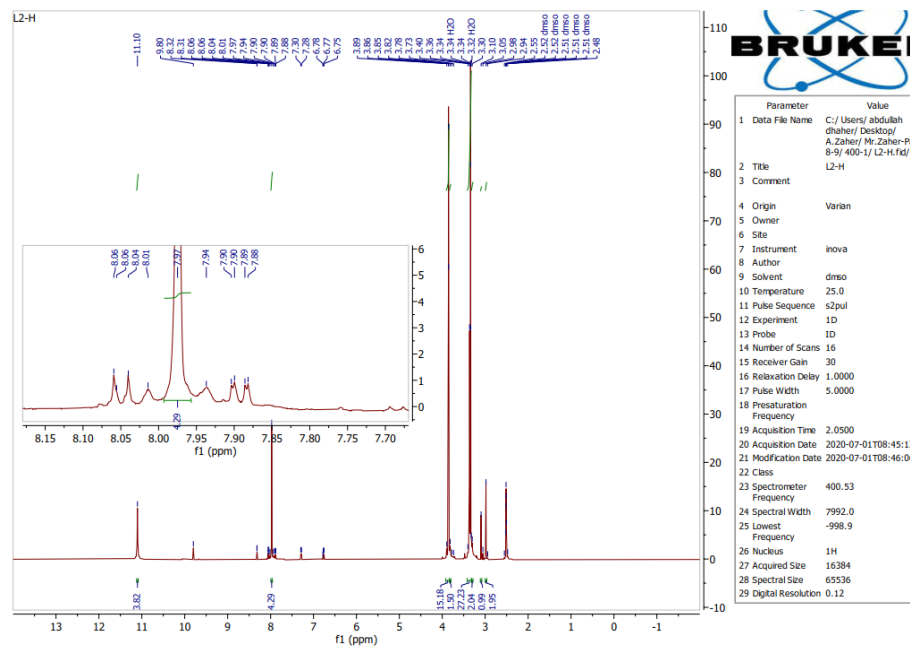
Figure(11):Thermogram for the $[\text{La}(\text{PAC})_2]\text{Cl}$ complex



Figure(12):Thermogram for the $[\text{Eu}(\text{PAC})_2]\text{Cl}$ complex

HNMR

The HNMR spectrum for ligand[Figure(13)] was recorded in DMSO with (TMS) as an internal standard. The spectrum of the ligand (PAC) was appeared the main signals at ($\delta=10$ ppm,H), ($\delta=9.8$ ppm,H) and ($\delta=8.06\text{--}7.88$ ppm,10H) were related to (NH amide), (-OH phenol), and (H pyrimidine), and benzene moiety respectively [17].



Figure(13):¹HNMR Spectrum for the (PAC) ligand

Biological activity

The ligand (PAC) and its complex containing heterocyclic moiety, where they gave them biological importance with high effectiveness for the treatment of many diseases [22]. In this study the synthesized compound were assessed for their antibacterial activity against four different pathogenic strain, two Gram positive (*Staphylococcus Aureas*) and (*Bacillus subtilis*) and two Gram negative (*Escherichia Coli*) and (*Pseudomonas aeruginosa*) and *Candida albicans* as fungi. The ciprofloxacin and clotrimazole standard drug as compared. All data is illustrated in Table (5). All the synthesized compounds have higher antibacterial and antifungal activity. Most lanthanide complexes have higher activity than free ligand. This due to the bases of overtones concept and chelation theory [23]. On coordination, the polarity of the lanthanide ion is reduced to greater scope due to the over-ligand orbital and partial sharing of the lanthanide ion's positive charge with the donor groups, which increase the delocalization of the p- and f- electrons over the chelates and supports the lipophilicity of the complex. Additionally, lipophilicity aids in complex penetration into lipid membranes and hinders metal binding to microorganism enzymes [24].

Table(5): Biological activity of the ligand (PAC) and its complexes

Compounds	Gram negative		Gram Positive		Candida albicans
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	
Ethanol	----	----	----	----	---
Ciprofloxacin	30	30	30	----	30
Clotrimazole	----	----	----	17	----
PAC	35	32	30	30	32
[La(PAC) ₂]Cl	39	36	38	38	36
[La(PAC) ₂]Cl	42	40	44	43	46
[La(PAC) ₂]Cl	40	38	42	45	44

The cytotoxic effectiveness

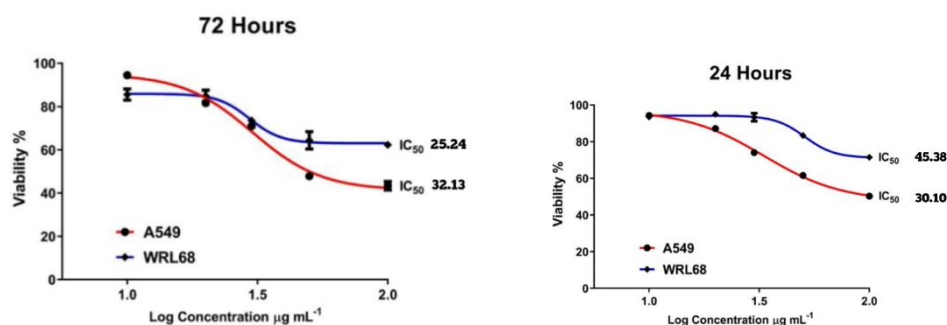
Cancer remains the second cause of death worldwide, despite of there are various drugs for the treatment of cancer, due to limitations such as high toxicity and adverse side effect [10,25]. For this purpose there is a need to novel anticancer drugs with less toxicity and side effect. In this study the cytotoxic effect of [Nd(PAC)₂]Cl complex against lung cancer cell line (A549) and normal cells (WRL-68) were investigated at different concentration (10,20,30,40,50,100) $\mu\text{g/ml}$ for (24 hrs. and 72 hrs.) by utilizing cell viability assay (MTT assay) [15]. Figures (14) and Table (6 and 7) are shown the results were obtained.

The [Nd(PAC)₂]Cl complex has cytotoxic effect at the concentration (100 $\mu\text{g/ml}$) after incubation (24 hrs.) [49.691 $\mu\text{g/ml}$] for tumor cell death, while at the same concentration after incubation at (72 hrs.) [56.622 $\mu\text{g/ml}$] with very good IC₅₀ (30.1 and 32.13) $\mu\text{g/ml}$ respectively. At the same time the effect of the complex on normal cells (WRL-68) at the same concentration and time of incubation is equal to (25.24 $\mu\text{g/ml}$ for 24hrs and 37.648 $\mu\text{g/ml}$ for 72hrs) with IC₅₀ (45.38 and 25.24) $\mu\text{g/ml}$. we conclude from the above that the effect of the properties of each the drug (complex) and cancer cell, which induced cell death as well as DNA damages, this causes a metal ion to bind with DNA at the (N7) location of two guanine or guanine and adenine bases preventing transcription and blocking DNA replication [26].

Table (6):Evaluation of cytotoxicity for [Nd(PAC)₂]Cl complex against lung cancer cell line after incubation(24hrs) and (72hrs) at (37°C)

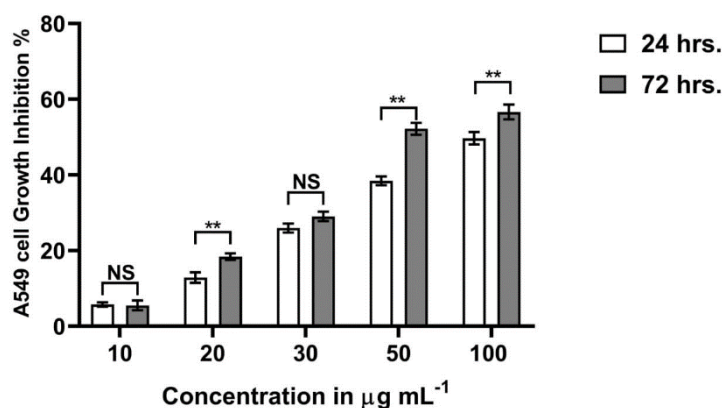
Cell line	Concentration $\mu\text{g/ml}$					Number of value	IC ₅₀ $\mu\text{g/ml}$	P-value
	10	20	30	50	100			
A549	94.252 ± 0.583	87.114 ± 1.381	74.035 ± 1.180	61.574 ± 1.140	50.309 ± 1.622	3	30.1	0.0001<
WR68	93.480 ± 1.142	94.946 ± 0.241	93.325 ± 2.188	83.372 ± 1.492	71.489 ± 1.395	3	45.38	

Cell line	Concentration $\mu\text{g/ml}$					Number of value	IC ₅₀ $\mu\text{g/ml}$	P-value
	10	20	30	50	100			
A549	94.499 ± 1.286	81.602 ± 0.834	70.965 ± 1.217	47.816 ± 1.566	43.378 ± 1.954	3	32.13	0.0001<
WR68	85.571 ± 2.589	89.915 ± 2.675	73.534 ± 1.094	64.352 ± 4.051	62.352 ± 1.417	3	25.24	



After incubation (72hrs)at(37°C)

C4

**Figure (14): Cytotoxicity effect of [Nd(PAC)₂]Cl on lung cancer and normal cell line after incubation for (24 and 72)hrs.**

Dyeing properties

The ligand (PAC) and its complexes have good dyeing ability, where they were used to dye cotton fabrics and study the extent of their stability towards rubbing and washing [Table (8)] and Figure (15)].The data was compared to gray scale according provided colors in the range of orange, purple, violet, red with depth on the fabric and good brightness. According to Iraqi specifications[NO.3616] for woolen textile. These dyes provided colors in the range of orange, purple, violet, and red with depth on the fabric and good brightness[7,8].

Table (8):Dying data for the ligand (PAC)and their complexes

Compound	Color	Textile color fastness to wet and dry abrasion		Textile color fastness check for washing	
		dry rubbing	wet rubbing	Staining with dye	Color change
PAC	Orange	5	5	4	3
[La(PAC) ₂]Cl	Purple	4	5	3	5
[Nd(PAC) ₂]Cl	Red	5	4	5	5
[Eu(PAC) ₂]Cl	Violet	5	3	5	4

(4-5)good ,(3)moderate and (1-2) not good

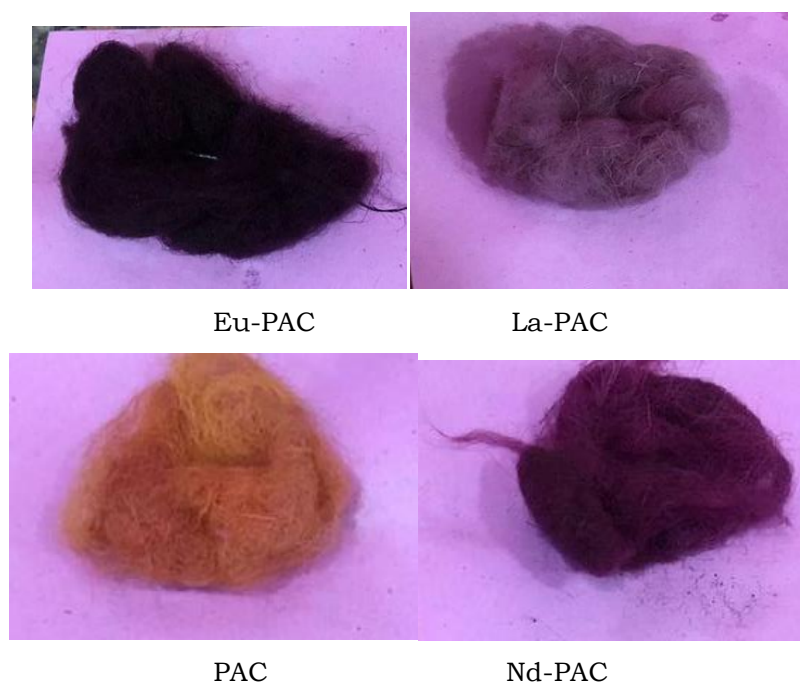


Figure (15): Dying of the ligand (PAC) and it's complexes

Conclusion

In this work study the synthesis of novel azo ligand (PAC) derived from cytosine by conventional diazo-coupling reaction .Then the ligand and its complexes were characterized by different spectroscopic methods. The stoichiometry of all complexes are (1:2)(M:L) and they have octahedral geometry. The ligand and its complex were appeared good deactivating capacity against pathogenic strains and similarly, the anticancer activity of $[\text{Nd}(\text{PAC})_2]\text{Cl}$ against A549 cell line was established to have potential anticancer. Fine shiny colors were appeared by testing dyeing performance of the ligand (PAC) and its complexes on wool fabric.

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